and sodium channel function at equipotent clinically relevant concentrations. Effects on lipid bilayer properties were tested using a gramicidin channel based stopped-flow fluorescence assay for lipid bilayer perturbation; effects on sodium channel function were tested using whole-cell voltage-clamp electrophysiology on neuronal cells (ND7/23). The stopped-flow results showed that all four FBs minimally affected lipid bilayer properties, whereas the sodium channels were strongly inhibited by all four anesthetics. Inhibition of peak sodium current was voltage-dependent as a pre-pulse to a voltage at which half the channels were in the fast inactivated state ($V_{1/2}$) revealed strong inhibition compared to a pre-pulse to a voltage at which the majority of the channels were in the resting state (V_0). The FBs produce a left-shift in the voltage of half-maximal inactivation ($V_{1/2}$, also known as h_∞ or availability), with 1,2-DiFB showing the greatest and HexaFB the least shift; these changes are comparable to those observed with modern inhaled anesthetics such as isoflurane. Together these results suggest that these compounds alter sodium channel function through direct interactions with the channels, though we cannot exclude that membrane effects may become involved at high, supra-pharmacological concentrations.

2196-Pos Board B215

Relationship between Membrane Fluidity Changes, Phospholipid Protrusion Probability and Phospholipase A_2 Activity during Thapsigargin-Induced Apoptosis

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Secretory phospholipase A_2 exhibits much greater activity toward apoptotic versus healthy cells. Various plasma membrane changes responsible for this phenomenon have been proposed, including biophysical alterations described as ''membrane fluidity'' and ''order.'' Understanding of these membrane perturbations was refined by applying studies with model membranes to fluorescence measurements during thapsigargin-induced apoptosis of S49 lymphoma cells using probes specific for the plasma membrane: Patman and trimethylammonium-diphenylhexatriene. Alterations in emission spectra or anisotropy of these probes corresponded with enhanced susceptibility of the cells to hydrolysis by secretory phospholipase A_2. Furthermore, these alterations appeared to correlate temporally with fragmentation of actin filaments detected by confocal microscopy of phalloidin fluorescence. By applying a quantitative model, additional information was extracted from the kinetics of Patman equilibration with the membrane. Taken together, these data suggested that the phospholipids of apoptotic membranes display greater spacing between adjacent headgroups, reduced interactions between neighboring lipid tails, and increased penetration of water among the heads. The phase transition of artificial bilayers was used to calibrate quantitatively the relationship between probe fluorescence and the energy of interlipid interactions. This analysis was applied to results from apoptotic cells to estimate the frequency with which phospholipids protrude sufficiently at the membrane surface to enter the enzyme's active site. The data suggested that this frequency increases 50-100-fold as membranes become susceptible to hydrolysis during apoptosis.

2197-Pos Board B216

Differential Oligomeric Nature of mEos2 and mEos3.2 Fluorescent Proteins is Consequential to Diffusion and Confinement of Membrane Probes

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Lipid-mediated membrane heterogeneity is proposed to be an important organizing principle in mammalian cells. using super-resolution fluorescence localization imaging we track the diffusion of a large panel of fluorescent membrane probes ranging in size, mode of membrane anchoring, and putative phase-association. Our expressed probes include fluorescently-tagged palmitoylated or non-palmitoylated versions of transmembrane domains including linker of activated T-cell and haemagglutinin, and fluorescent protein anchored by GPI, palmitate-myristoyl moieties, or a geranyl-geranyl moiety. The recent advent of photoconvertible fluorescent protein mEos3.2, a ''truly monomeric'' mutant of its mEos2 predecessor1, has enabled us to compare directly monomeric and oligomeric versions of the same probes. Our results indicate that in some cases rates of diffusion are more than two fold lower in the mEos2 probes when compared to their mEos3.2 counterparts. Many mEos2-labeled probes also differ from mEos3.2-labeled probes in their exponents of anomalous diffusion. We attribute changes in mobility to the proteins' differential propensity to oligomerize, and are using the clustering effect of mEos2 to investigate whether phase heterogeneity influences cluster mobility.

1 Zhang, M. et al. Rational design of true monomeric and bright photoactivatable fluorescent proteins. Nat Methods 9, 727-729. (2012).

2198-Pos Board B217

Super-Resolution Imaging of T Cell Triggering Supports the Kinetic Segregation Model in the Adaptive Immune Response

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Among the most contentious questions in immunology is: how is the T cell receptor (TCR) triggered? using a combination of different single-molecule tracking, novel calcium signalling assays, crystallography and super-resolution imaging techniques we have demonstrated evidence to support the kinetic segregation model as the molecular mechanism behind the adaptive immune system.

The phosphorylation of the T cell receptor is required for T cell activation. The kinetic segregation model predicts that this is achieved by the spatial partitioning of important components such as the TCR and Lck kinase from the CD45 phosphatase according to the difference in size of the extracellular domain of the proteins on the cell membrane. We test this prediction by imaging the formation of contacts between T cells with a model antigen presenting cells in early stage contacts at endogenous expression levels. The approach allows the interrogation of the kinetic segregation with other models based upon aggregation or conformation change of the TCR. We present the results of this study.

2199-Pos Board B218

Properties of Membranes Derived from the Total Lipids Extracted from the Human Lens Cortex and Nucleus

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The organization and physical properties of lens lipid membranes made from the total lipids extracted from the human lens cortex and nucleus of 41- to 60-year-old donors were investigated using electron paramagnetic resonance (EPR) spin-labeling. Profiles of the phospholipid alkyl-chain order, fluidity, oxygen transport parameter, and hydrophobicity were assessed across membranes and coexisting membrane domains. Lens lipid membranes prepared from the lens cortex and nucleus were found to contain two distinct lipid environments, which were assigned as the bulk phospholipid-cholesterol domain and the cholesterol bilayer domain (CBD). The alkyl chains of phospholipids were strongly ordered at all depths, indicating that the amplitude of the wobbling motion of alkyl chains was small. However, profiles of the membrane fluidity, which explicitly contain time (expressed as the spin-lattice relaxation rate) and depend on the rotational motion of spin labels, show relatively high fluidity of alkyl chains close to the membrane center. Profiles of the oxygen transport parameter and hydrophobicity have a rectangular shape with an abrupt change between the C9 and C10 positions, which is approximately where the steroid ring structure of cholesterol reaches into the membrane. The amount of CBD was greater in nuclear membranes than in cortical membranes. The presence of the CBD in lens lipid membranes, which at 37°C showed a permeability coefficient for oxygen about 60% smaller than across a water layer of the same thickness, would be expected to raise the barrier for oxygen transport across the fiber cell membrane.

Protein-Lipid Interactions II

2200-Pos Board B219

Structural and Dynamical Changes for different types of Lipid Bilayer by Different Length of Poly-L-Lysine: MD Simulations

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Poly-L-lysine (PLL), a cationic polypeptide, is toxic to a variety of cell types (Nano letters 8, 2008; Nano letters 5, 2005) and is also an ideal vector for gene delivery (Pharmaceutical Research 17, 2000;Molecular Therapy 6, 2002) by forming pore in cell membrane and letting small molecules passing through membrane with it. Molecular mechanisms for the controversial role of PLL in cell membrane remain unclear. We investigated the molecular interactions of poly(L-lysine) at two different lengths with three different types of lipid bilayer: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS) and

mixed POPC and POPS bilayer with molecular dynamics (MD) simulations. One short PLL with more definite conformation and one relative long PLL with random conformation were used for this study. With the simulated systems reached dynamic equilibration, additional 200 ns production MD simulations were performed for each simulated systems. Results demonstrated that the interactions of different length of PLLs with the lipid bilayer directly affect the microscopic properties of a lipid bilayer: lipid head group area, tail order and bilayer thickness, further influencing the mesoscopic properties of the bilayer: electrostatic and mechanical properties of the bilayer. Results also showed that the different types of lipid bilayer can in turn influence the binding dynamics of PLLs with the lipid bilayer. The results will provide molecular insight for the experimental observations about the effect of PLL on cototoxicity and its role in gene delivery, further helping to optimize PLL length and lipid environment for best usage of PLL for its biological applications.

2201-Pos Board B220

Probing the Membrane Bound KCNE1 Protein with Solid State NMR Spectroscopy

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KCNE1, also known as MinK, is a membrane protein that associates with the KCNQ1 channel protein to form a voltage-gated potassium channel. This ion channel is essential to the cardiac action potential that mediates heartbeat and is also critical for potassium ion homeostasis in the inner ear. Dominant mutations in KCNE1 lead to congenital long-QT syndrome and congenital deafness. KCNE1 has been over expressed in E. coli, purified into micelles using his-tag affinity chromatography, and reconstituted into POPC/POPG vesicles. 31P NMR powder spectra results confirm vesicle formation. Different KCNE1 mutants have been labeled using MTSL, one mutant outside the membrane and the other inside the membrane. By measuring 31P relaxation times of the lipids, we can determine the depth that at which KCNE1 is buried inside the vesicles. We also introduced a bicelle system to study the topology of uniform 15N labeled KCNE1 with respect to the lipid bilayer. By measuring the 15N NMR signal, we are able to figure out the structural topology of KCNE1 within the lipid bilayer.

2202-Pos Board B221

Divalent Cation-Induced PIP2 Clustering in Cholesterol-Containing Membranes: How PIP2 Lateral Distribution affects PIP2-Gelsolin Interaction

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Phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2) is involved in many important cellular events, but the mechanism by which this relatively rare lipid selectively regulates specific proteins is unclear. While many proteins bind to PIP2 with similar affinities in vitro, only a small subset interact with PIP2 at specific sites during specific cellular processes. Previously, we showed that lateral distribution of PIP2 in a background of neutral lipids is affected by divalent cations through counterion-mediated attraction. We further hypothesize that spatial control of PIP2 concentration can alter the binding of this lipid to its protein targets. To test this hypothesis, we determine if Ca^{2+}-induced PIP2 clustering affects the interaction between PIP2 and PIP2-binding proteins with polybasic domains under liquid order/liquid disorder (Lo/Ld) phase demixing conditions.

We first characterize the effect of divalent cations on Lo/Ld phase-demixing. The addition of Ca^{2+} induces a surface pressure drop and lowers the transition surface pressure. In contrast, Mg^{2+} increases the transition surface pressure and has a minimum condensing effect. Topography measurements through AFM show that Ca^{2+}-induced PIP2-rich clusters co-localize with the Lo phase. The effect of PIP2 microdomain/cluster formation on the regulation of gelsolin was studied using an actin filament severing assay and Ca^{2+}-insensitive gelsolin fragments (NtGSN). This functional assay suggests that membrane partitioning of NtGSN is sensitive to PIP2 local concentration upon phase separation, which also depends on the temperature. Cholesterol-induced phase demixing strongly inhibits the severing function of gelsolin, and the presence of PIP2 clusters formed by micromolar Ca^{2+} also improves the inhibition efficiency. These observations suggest that changes in local PIP2 distribution might be a major mechanism to determine how proteins interact with PIP2 in the membrane. This research may shed a light in studying the interplay between PIP2, cholesterol and Ca^{2+}-signaling.

2203-Pos Board B222

Lipid Behavior in Integrin α2 Clustering

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It is becoming increasingly more evident that transmembrane proteins can influence lipid organization in biological membranes. Our previous studies in model membranes have shown that peptides affect the partitioning of lipids in the bilayer. We now want to further investigate these protein-lipid interactions in a more biological context, by looking at integrin clustering in giant plasma membrane vesicles (GMPVs). Integrins are cell surface proteins that traverse the plasma membrane and mediate, for example, cell signaling and virus entry. Binding of Echovirus 1 to integrin α2 induces integrin clustering and the subsequent internalization of the virus-integrin complex. Clustering is crucial for virus entry, but can also be achieved through the addition of integrin specific antibodies to cells. We are interested in the possible reorganization of the membrane upon clustering as well as the influence of plasma membrane lipids on clustering. For this purpose we have prepared GMPVs from SAOS cells expressing α2 integrin, and induced antibody-mediated clustering in them. We are using lipid probes to visualize the liquid ordered and liquid disordered phase, and fluorescent secondary antibodies to detect α2 integrin in the GPMVs with confocal microscopy. We will look at lipid organization in cells expressing integrin mutants, which are unable to cluster and/or internalize. And we will treat the cells and/or GPMVs in ways that alter their lipid content, such as deplete membrane cholesterol with cyclodextrin, and monitor the resulting changes in integrin and lipid behavior in GMPVs. With these experiments we hope to gain further insight into the interplay of transmembrane proteins and lipids in biological membranes. Also, understanding the lipid behavior in integrin clustering will aid in understanding what governs clustering and how it affects membrane architecture. This information can in turn be beneficial for developing ways of preventing virus entry.

2204-Pos Board B223

Surface Reflectivity from Absorbed Films of Pulmonary Surfactant at the Air-Liquid Interface

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Pulmonary surfactant (PS) is the complex mixture of lipids and proteins that forms a thin film on the liquid layer that lines the alveolar air-sacks of the lungs. When compressed by the decreasing alveolar surface area during exhalation, the surfactant films reduce surface tension to exceptionally low levels. This behavior in situ contrasts with the performance of spread monolayers in vitro that contain the complete set of surfactant constituents, which collapse promptly when compressed below the equilibrium spreading pressure. The structural characteristics that provide the basis for this functional difference remain controversial. The studies here used X-ray reflectivity to compare the structure of adsorbed films and spread monolayers of extracted calf surfactant on the air-water interface. Our results show the presence in the adsorbed film of additional double layers of distinct electron densities underneath the interfacial monolayer. These results support previous evidence that adsorbed films of pulmonary surfactant have multilamellar thickness, and that the additional layers may have functional significance.

2205-Pos Board B224

Thionaphthoquinones Destabilization of Phospholipid Bilayers

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Quinones are structures present in many naturally occurring compounds, e.g. 1,4-naphthoquinones like Vitamin K, doxorubicin, etc. are among the examples of this vast class of chemicals used in the treatment of bleeding, lymphoma, carcinoma, etc. Only one sulfurated naphthoquinone was found in nature but many were synthesized and proved to be potent antibacterial and antifungal agents. Furthermore, several thionaphthoquinones have been recently synthesized because of their interesting spectroscopic properties and also as attractive organic dyes due to their high solubility in organic solvents. their red color in the solid state also leads to applications as organic nonlinear optical materials. New thionaphthoquinones and hydroquinones, bearing alkyl side chains that match the phospholipids POPC and POPE, were synthesized in order to investigate their interactions with lipids. It was observed that, in general, these additives destabilize the lipid bilayer and induce less organized structures with higher amount of curvature. Moreover, cubic phases, not normally observed in the pure lipids when fully hydrated, were detected. Coexistence of lamellar phases was interpreted as a consequence of microsegregation of the components in the mixtures